THE SYSTEMATICS OF *GASTEROSTEUS ACULEATUS* (PISCES: GASTEROSTEIDAE) IN CENTRAL AND SOUTHERN CALIFORNIA[1]

By Stephen T. Ross[2]

Abstract: Morphological, breeding, and chromosome studies of the three principally allopatric forms of *G. aculeatus*: the anadromous *G. a. aculeatus* and the freshwater *G. a. microcephalus* and *G. a. williamsoni* are presented. Emphasis is primarily on the two latter forms. Combined population samples of the *williamsoni* form are morphologically distinct from combined samples of *microcephalus* with 96% joint nonoverlap of character index values. Reciprocal crosses between *G. a. williamsoni* and *G. a. microcephalus* do not conclusively demonstrate postmating isolating mechanisms, although hybrid crosses show only 69% fertilization compared to 90% for the controls. There are no statistically significant differences in mortality between control and test crosses for either embryos or larvae. Positive identification of laboratory reared F_1 hybrids by plate number, spine length, or by a character index is not possible due to dominance of *G. a. microcephalus*. The value of plate counts in the determination of wild F_1 hybrids is thus questionable. *G. a. aculeatus, G. a. microcephalus,* and *G. a. williamsoni* all have a diploid chromosome number of 42. Retention of the subspecific nomenclature is favored.

Introduction

The threespine stickleback, *Gasterosteus aculeatus* Linnaeus, displays complex patterns of variation throughout its distribution. Populations of *Gasterosteus* on the Pacific Coast of North America have recently been subjected to considerable research by Hagen (1967), McPhail (1969), Miller and Hubbs (1969), Narver (1969), Hagen and McPhail (1970), and Hagen and Gilbertson (1972). Hagen and McPhail discussed the extreme variability of many populations and presented and reviewed evidence calling for specific recognition of *G. trachurus* and *G. leiurus*. In contrast, Miller and Hubbs (1969) supported retention of the subspecies *G. a. aculeatus, G. a. microcephalus,* and *G.a. williamsoni,* as suggested by Jordan and Hubbs (1925). Most of the problems discussed by Hagen and McPhail pertain to stickleback populations in the Pacific Northwest. For the purpose of this investigation, centered in central and southern California, the nomenclature proposed by Jordan and Hubbs (1925) will be followed. However, as stated by Hagen and McPhail (1970), "A revision of the nomenclature may be necessary once the data are in hand."

This study attempts to clarify certain relationships of the central and southern California populations of sticklebacks.

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NOMENCLATURE AND DISTRIBUTION

This section discusses the literature dealing with the systematics of *G. a. williamsoni* in southern California. First locality records from an area are cited, but additional records from the same area are omitted unless they conflict with the original identifications or contain additional information. References to *G. a. microcephalus* are restricted to major papers. A listing of the earlier literature is given by Eigenmann (1886: 245) and Evermann and Clark (1931).

Gasterosteus aculeatus williamsoni Girard

Gasterosteus williamsoni Girard, 1854: 133. (original description; type locality, Williamson's Pass, determined by Miller (1960) to be Soledad Canyon of the Santa Clara River, Los Angeles County, California).

Eucalia williamsoni, Jordan and Copeland, 1876: 141. (identification doubtful as to genus; Williamson's Pass, California).

Gasterosteus williamsoni, Gilbert, 1893. (possible subspecific recognition; San Bernardino County, California). Jordan and Evermann, 1896: 750. (characters; abundant in southern California).

Gasterosteus williamsoni williamsoni, Rutter, 1896: 245. (survey of Pacific slope populations; "True *williamsoni*" from Santa Ana basin at Colton, Riverside and San Bernardino, San Bernardino County, California. Also eight type specimens from Williamson's Pass, Santa Clara River).

Gasterosteus santaeannae. Regan, 1909: 345. (original description; type locality, Santa Ana River, Colton, California).

Gasterosteus cataphractus, Snyder, 1913: 17. (characters; examination of Rutter's (1896) data).

Gasterosteus aculeatus williamsoni, Jordan and Hubbs, 1925: 200. (synonymy — *cataphractus* = *aculeatus; santaeannae* = *williamsoni*; Santa Ana drainage in southern California). Hubbs, 1929: 4. (plate II, figure 2). Miller, 1939: 168. (recorded from San Juan Creek and Arroyo Trabuco, Orange County, California). Miller, 1960: 350. (characters; San Juan Creek and Arroyo Trabuco samples do not represent unarmored form, also recorded from Alamo Creek and Huasna River in Santa Maria River drainage, Santa Barbara County, California). Miller, 1961: 388. (Los Angeles River, San Gabriel River and Mohave River (introduced)). Miller and Hubbs, 1969: 52. (characters, plate counts for southern California populations).

Distribution. Santa Clara River in Soledad Canyon, Santa Ana[3], San Gabriel[3], Los Angeles[3], and Mohave rivers, and Alamo Creek and the Huasna River (Santa Maria River drainage). Miller and Hubbs (1969) reported *G. a. microcephalus* x *williamsoni* intergrades introduced in the Mohave River, and *G. a. microcephalus* introduced into Alamo Creek and the Huasna River.

[3]Probably extirpated between 1942-1961 (Miller, 1969)

Gasterosteus aculeatus microcephalus Girard

Gasterosteus microcephalus Girard, 1854: 133. (original description; type locality, Four Creek (Kaweah River), San Joaquin Valley, California). Eigenmann, 1886: 245. (characters; distribution from Bering Strait to Todos Santos Bay). Jordan, 1894: 141. (San Luis Obispo County).

Gasterosteus williamsoni microcephalus, Rutter, 1896: 245. (characters; list of synonyms; distribution from Unalaska to Santa Clara River in southern California). Jordan and Evermann, 1896: 751. (characters; distribution, Alaska to Todos Santos Bay).

Gasterosteus cataphractus, Jordan and Gilbert, 1899: 443. (intergrades into fully plated form and thus lacks characters of a subspecies). Evermann and Goldsborough, 1906: 273. (distribution in Alaska). Snyder, 1913: 71. (distribution: streams tributary to Monterey Bay).

Gasterosteus aculeatus, Regan, 1909: 435. (characters; synonymy of European and American forms).

Gasterosteus aculeatus microcephalus, Jordan and Hubbs, 1925: 202. (distribution: California, Aleutian Islands and Japan). Miller and Hubbs, 1969. (characters; plate counts for Pacific Coast populations).

Gasterosteus aculeatus, Hagen, 1967: 1637. (characters and isolating mechanisms of British Columbia populations).

Gasterosteus leiurus, Hagen and McPhail, 1970: 147. (discussion of isolating mechanisms).

Distribution. Freshwater of Pacific Coast of North America from Bering Strait (Eigenmann, 1886) to Arroyo del Rosario, Baja California (Hubbs, *in* Follett, 1960), with a hiatus in its range between the Santa Clara River and San Juan Creek (Miller and Hubbs, 1969: 63).

Material Examined

The localities, numbers of specimens, dates of collection, and museum numbers are given below (see also Fig. 1). Material without museum numbers is at California State College, Fullerton. Santa Cruz County: San Lorenzo River (5), 10 July, 1957, California Academy of Sciences 26738; San Lorenzo River (10), 3 April, 1969, Los Angeles County Museum, LACM 30905-1. Monterey County: Carmel River (30), 17 June, 1966, LACM 30889-1; Big Sur River (15), 19 June, 1966, LACM 30907-1; San Antonio River, Hunter-Liggett Military Reservation, (25), 25 February, 1968; Nacimiento River, below Nacimiento Dam (11), 25 February, 1968. San Luis Obispo County: Toro Creek (12), 2 July, 1968, LACM 30916-1; Morro Creek (30), 26 December, 1967, LACM 30888-1; San Luis Obispo Creek, in San Luis Obispo, (25), 26 December, 1967, LACM 30909-1; San Luis Obispo Creek (11), 21 January, 1968, LACM 30927-1; San Luis Obispo Creek (23), 25 February, 1968, LACM 30923-1; San Luis Obispo Creek (25), 27 June, 1968, LACM 30894-1; Price Creek (29), 22 June, 1968, LACM 30938-1; Arroyo Grande Creek (30), 20 August, 1967, LACM 30904-1; Alamo Creek (10), 9 December, 1967, LACM 30897-1; Alamo Creek (17), 25 February, 1968, LACM 30932-1. Santa Barbara

County: Salsipuedes Creek (27), 20 August, 1967, LACM 30911-1. Ventura County: Ventura River at Foster Park (30), 15 September, 1967; Piru Creek, below Piru Dam (5), 15 September, 1967, LACM 30908-1; Santa Clara River near the Los Angeles-Ventura County line (25), 8 July, 1968, LACM 30937-1. Los Angeles County: Santa Clara River in Soledad Canyon, west of Bee Canyon (44), 15 September, 1967, LACM 30892-1; Santa Clara River, Soledad Canyon (25), 27 June, 1968, LACM 30925-1. San Bernardino County: Mohave River, at Las Flores junction, Summit Valley (30), 18 July, 1966, LACM 30890-1. Orange County: Arroyo Trabuco, near Mission San Juan Capistrano (30), 15 January, 1968.

Experimental studies were conducted with fish from Soledad Canyon of the Santa Clara River, Los Angeles County (the type locality of *G. a. williamsoni*), and San Luis Obispo Creek, San Luis Obispo County. Also, a male *G. a. aculeatus* from Scott Creek, Santa Cruz County, was utilized in breeding studies and individuals of *G. a. aculeatus* from the San Lorenzo River, Santa Cruz County, were karyotyped.

METHODS

Meristic characters studied were the numbers of lateral plates, gill rakers, and vertebrae. Dorsal, anal, and pectoral rays were not counted since Heuts

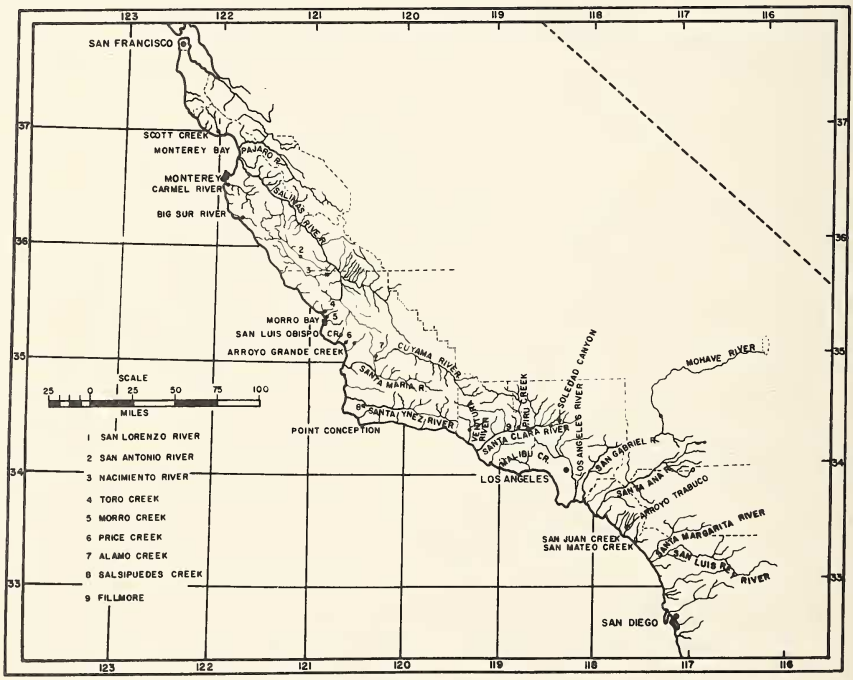

FIGURE 1. Coastal drainages in central and southern California. The collecting sites are indicated by small circles.

(1949), Lindsey (1962), and Hagen (1967) have shown fin rays to be influenced greatly by water temperature. Dial calipers read to the nearest 0.1 mm were used in obtaining all morphometric data from preserved specimens. Magnification was used whenever necessary, generally with specimens under 25 mm SL and with larger *G. a. williamsoni* in determining spine length.

Measuring and counting techniques followed Hubbs and Lagler (1958) with the following modifications. All lateral plates were counted, including those in the peduncular keel (when present), by using an air jet and a dissecting microscope. Small individuals were first stained with alizarin to aid plate enumeration. All gill rakers on the first gill arch were counted; determination of rudimentary rakers was aided by staining the arch (in situ) with an aqueous solution of methylene blue (1:5,000). Vertebral counts (determined from radiographs) included the urostyle and the expanded hypural plate as one, and the first vertebra was regarded as the one to which the first neural spine attached. Orbit diameter was measured between the anterior and posterior bony margins. Body depth was measured anterior to the pelvic spines to avoid distortion in gravid females. Body width was measured anterior to the pectoral fins. Dorsal and pelvic spine lengths were taken along the anterior surfaces. Pubic-plate length (ventral shield, Nelson, 1971) was measured from a point between the anterior face of the insertions of the erected pelvic spines and the end of ossification. All morphometric data are presented as proportions with standard length as the numerator.

Peduncular depth, first and second dorsal spine lengths, pelvic-spine length, pubic-plate length, and the number of lateral plates and gill rakers were combined in a character index using the following procedure. The ranges of the proportions formed from the three spine lengths and pubic-plate length were subdivided into 20 equal classes which were scored from 0 to 10 with increments of 0.5. A score of zero was reserved for situations in which the character was missing, but zero values were not used in determining the index which is the mean of the scored characters. Character states typical of *G. a. williamsoni* received the lowest scores, while character states typical of *G. a. aculeatus* received the highest scores. Lateral-plate number was not divided into 20 equal classes due to the extreme range. Counts of zero to seven, such as characterized freshwater populations of *Gasterosteus,* were accorded individual classes; thereafter, two or three plate counts made up a class. Ranges in plate counts in which no recorded individuals fell were lumped in groups of three. Scoring of classes was the same as described previously.

Reciprocal crosses between *G. a. microcephalus* and *G. a. williamsoni* were incubated at 5, 10, 12, 15, 20, 25, and 30°C. Late in the study a female *G. a. williamsoni* was crossed with a male *G. a. aculeatus* at 20°C.

Artificial fertilization was produced following the technique of Hagen (1967). The four masses of fertilized eggs resulting from the two reciprocal crosses were each washed onto a platform of fiberglass screen supported by a ring of surgical tubing. Each platform was placed in one of four 1.1 liter

jars which extended 6 cm above the water in a 76 liter tank. Water was air-lifted from the large tank to a reservoir 8 cm above the water and then returned through 1.1 liter jars to the main tank via siphons. Complete exchange of water in the 1.1 liter jars occurred every 12 minutes. The jars were removable so that eggs and larvae could be counted by placing the jar over a grid. Eggs were kept in darkness prior to hatching and then received an irregular long-day photoperiod. Infusoria tablets were introduced three days after hatching, and after 10 to 12 days the larvae were fed washed nauplii of *Artemia salina.* Sticklebacks of 10 mm and larger were fed live immature and adult *A. salina,* adult frozen *A. salina,* and commercial dried food. Fish were kept in the controlled temperature systems for a minum of five months at which time the survivors were placed in 19 or 38 liter aquaria. After seven to nine months fish were fixed in 10% formalin for one to two weeks, washed and placed in 40% isopropanol.

Only laboratory reared sticklebacks larger than 20 mm SL were utilized. Characters studied were plate number, first and second dorsal and pelvic spine lengths, and pubic-plate length. Gill rakers were omitted due to the large amount of overlap between natural populations of *G. a. williamsoni* and *G. a. microcephalus,* and the depth of the caudal peduncle was omitted since it was not useful in separating hybrids and controls. The term "hybrid," as used in this paper, refers to crosses between different populations and does not imply an interspecific cross.

Temporary chromosome preparations were made following McPhail and Jones (1966), and permanent slides were made with an air drying method modified from Ojima et al. (1964), using the same pretreatment. Gill arches were fixed in 3:1 ethanol-acetic acid (20 minutes), brushed across a microscope slide and dried over an alcohol flame. The dried preparation was flooded with 3% aceto-orcein for five minutes, rinsed in distilled water, dried, and mounted with diaphane. Chromosomes were counted from photographic enlargements.

RESULTS

Morphological Studies

Statistical comparisons of lateral-plate numbers for 18 stickleback populations using Tukey's w′ procedure (Steele and Torrie, 1960) show three distinct groups (Fig. 2). The large middle group shows considerable intragroup overlap, and the variation follows a north-south cline only approximately. The three groups correspond with the subspecies *G. a. aculeatus, G. a. microcephalus* and *G. a. williamsoni* as defined by Miller and Hubbs (1969); however, the Arroyo Trabuco population will be shown to tend less toward *G. a. microcephalus* in certain other characters.

Gill rakers differ significantly in number between the 18 sample populations ($p<0.005$), and show an irregular decrease from north to south (Table 1). However, Tukey's w′ indicates a high degree of overlap between the means so that a statistical separation into taxonomically useful groups can-

SAN LORENZO RIVER	14	30.5
BIG SUR RIVER	15	8.3
TORO CREEK	12	7.1
CARMEL RIVER	30	6.5
MORRO CREEK	30	6.5
ARROYO GRANDE CREEK	30	5.4
PRICE CREEK	29	5.2
SALSIPUEDES CREEK	25	5.2
VENTURA RIVER	30	4.9
NACIMIENTO RIVER	10	4.9
SAN ANTONIO RIVER	25	4.7
SAN LUIS OBISPO CREEK	84	4.6
ARROYO TRABUCO	30	3.5
ALAMO CREEK	27	1.0
PIRU CREEK	7	1.0
SANTA CLARA RIVER 2	25	0.4
MOHAVE RIVER	30	0.4
SANTA CLARA RIVER 1	69	0.2

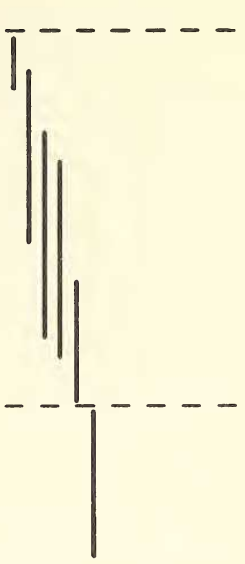

FIGURE 2. Sample sizes and ranked means of lateral-plate numbers from 18 stickleback populations. The means connected by the same line are not significantly different according to Kramers's (1956) modification of Tukey's w' multiple range test (a = 0.05). 1. Santa Clara River, Soledad Canyon. 2. Santa Clara River, Ventura County.

not be made. It should be noted that the use of multiple range tests modified for unequal sample sizes results in an increased probability of a significant difference occurring within a subset classified as homogeneous (Kramer, 1956).

Vertebral numbers from 12 sample populations do not show a definite geographic trend nor a trend congruent to variation in other characters studied (range 29-33).

Orbit diameter does not vary significantly among the populations sampled. However, the Santa Clara River, Salsipuedes, and Arroyo Grande creeks, and the San Antonio River samples display a tendency towards greater orbit size in some specimens. The variation in body depth also does not show statistically significant differences.

The ratio of standard length to body width decreases irregularly from north to south (Fig. 3a). Sample populations from the San Lorenzo, Carmel, and Big Sur rivers, and Toro, Morro, and Salispuedes creeks have more compressed bodies ($\bar{x}$ = 7.2; sd = 0.58). Plateless sticklebacks from the Santa Clara and Mohave rivers, and Alamo Creek have the roundest bodies ($\bar{x}$ = 6.61; sd = 0.59), but overlap is extreme with the southern partially plated sticklebacks.

The ratio of standard length to peduncular depth generally decreases from north to south although Nacimiento and San Antonio river samples have deeper caudal peduncles in comparison with sticklebacks of adjacent latitudes

TABLE 1
Counts of gill rakers in different populations of *Gasterosteus aculeatus* from southern and central California

Locality	12	13	14	15	16	17	18	19	20	21	22	23	24	25	N	$\bar{X}$
San Lorenzo River							2	2	3	3	5				15	20.5
Carmel River							3	3	7	13	4				30	20.3
Big Sur River							4		4	5	1	1			15	20.1
San Antonio River					3	2	7	6	6	1					25	18.5
Nacimiento River								7	1		2				10	19.7
Toro Creek							1	2	4	5					12	20.1
Morro Creek							3	9	4	11	2	1			30	20.1
San Luis Obispo Cr.					1	4	8	25	21	14	4	4	1	1	83	19.9
Price Creek							6	11	5	6	1				29	19.5
Arroyo Grande Creek			1				3	10	12	4					30	19.4
Alamo Creek				1	3	7	6	7	3						27	17.9
Salsipuedes Creek					1		3	9	5	2	3	2			25	19.8
Ventura River	1				7	7	9	5	1						30	17.3
Piru Creek					2	1		1							4	17.0
Santa Clara River, Soledad Canyon		3	4	2	18	19	14	8	1						69	16.8
Santa Clara River, Ventura County				1	5	4	11	4							25	17.5
Mohave River		1	2	2	6	13	1	3	2						30	16.8
Arroyo Trabuco		3	3	7	11	3	3								30	18.6

(Fig. 3b). This is also true of the Arroyo Grande sample. The Salsipuedes Creek sticklebacks have more slender caudal peduncles, but overlap is extensive with the adjacent populations.

The ratio of standard length to first dorsal spine and standard length to second dorsal spine show similar patterns of geographic variation (Fig. 3c, d). Samples from the San Lorenzo, Carmel, and Big Sur rivers, and Toro, Morro, San Luis Obispo, Arroyo Grande, Price, and Salsipuedes creeks, and the Ventura River have longer dorsal spines than other samples (I: $\bar{x}$ = 11.7; sd = 1.78; II: $\bar{x}$ = 10.1; sd = 1.43). Sticklebacks from Alamo Creek, the Santa Clara and Mohave rivers, and Arroyo Trabuco have the shortest dorsal spines (I: $\bar{x}$ = 19.1; sd = 3.82; II: $\bar{x}$ = 15.6; sd = 2.77). An intergroup separation with 91% joint nonoverlap is achieved with either spine . The San Antonio and Nacimiento river samples are intermediate between the two groups (I: $\bar{x}$ = 14.7; sd = 1.91; II: $\bar{x}$ = 12.9; sd = 1.62). The extremely short-spined Mohave River sample is statistically distinct from the Soledad Canyon sticklebacks

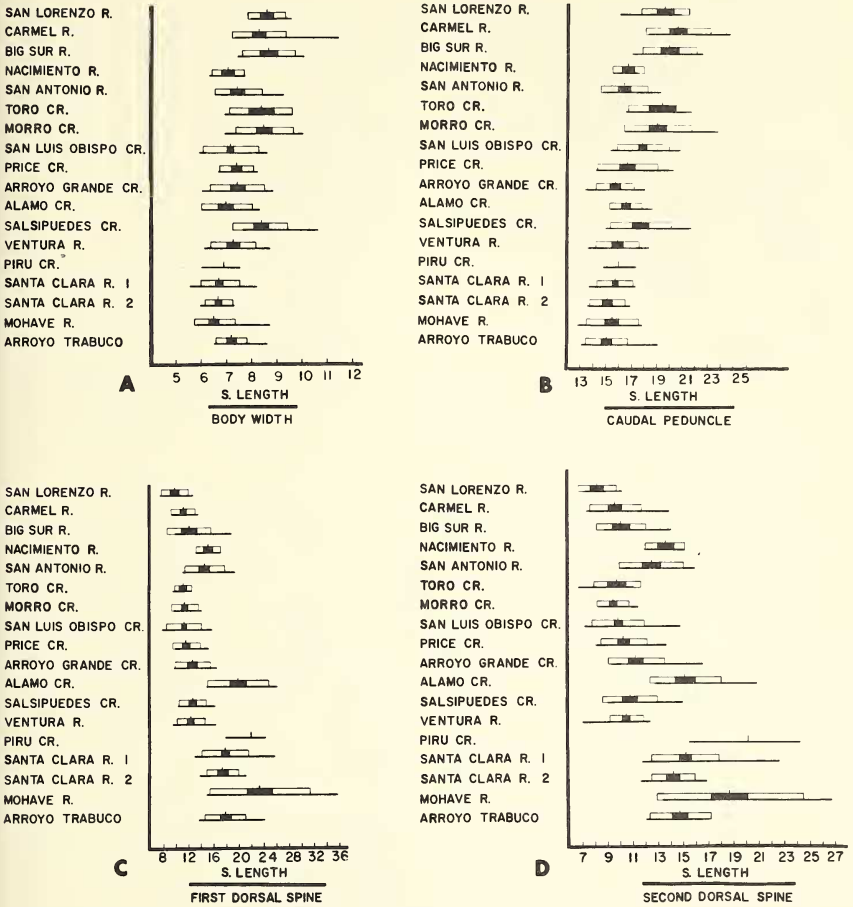

FIGURE 3. Morphometric variation of populations of *Gasterosteus aculeatus*. The diagrams indicate the mean (vertical lines), two standard errors on either side of the mean (closed rectangles), 1.5 standard deviations on either side of the mean (open rectangles), and the range (horizontal line).

($p<0.001$), although overlap is high. Variability for dorsal-spine lengths is extreme (CV=21-23).

The ratio of standard length to pelvic-spine length also distinguishes the same two groups listed above, with 92% joint nonoverlap (Fig. 4a). The San Antonio and Nacimiento river samples again show intermediacy although the former is closer to the long-spined group than the latter. The Big Sur sample ($\bar{x}$ = 8.5) is also less extreme in spine length, possibly due to the small sample size (15), and to the low mean standard length of the series. In specimens smaller than 19 mm there is a decrease in pelvic-spine length relative to standard length (Fig. 5a). The coefficients of variation are quite high for the Big

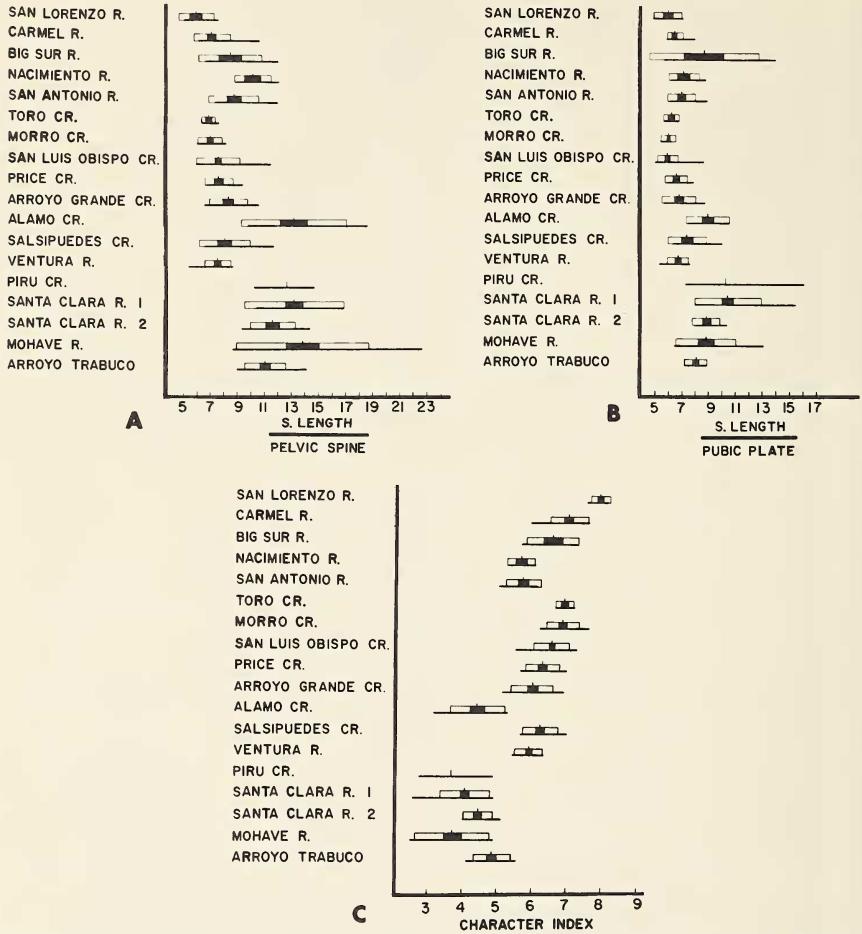

FIGURE 4. Morphometric variation and character index values of populations of *Gasterosteus aculeatus*. See legend for figure 3 for explanation of diagrams, and text for explanation of character index.

Sur sample (18.2), the samples from Alamo Creek (20.0), Soledad Canyon (19.0), and the Mohave River (22.5). Variation in the three latter populations is not a direct reflection of small size since almost all specimens were adults.

Variation in the ratio standard length to pubic-plate length (Fig. 4b) is quite similar to that shown by the dorsal and pelvic spines. The Big Sur sample shows high variability (CV = 32.0), which is again a manifestation of the small specimens and sample size. A decrease in pubic-plate length relative to standard length occurs between 20 and 22 mm (Fig. 5b). The San Antonio and Nacimiento river samples are not clearly separable from the

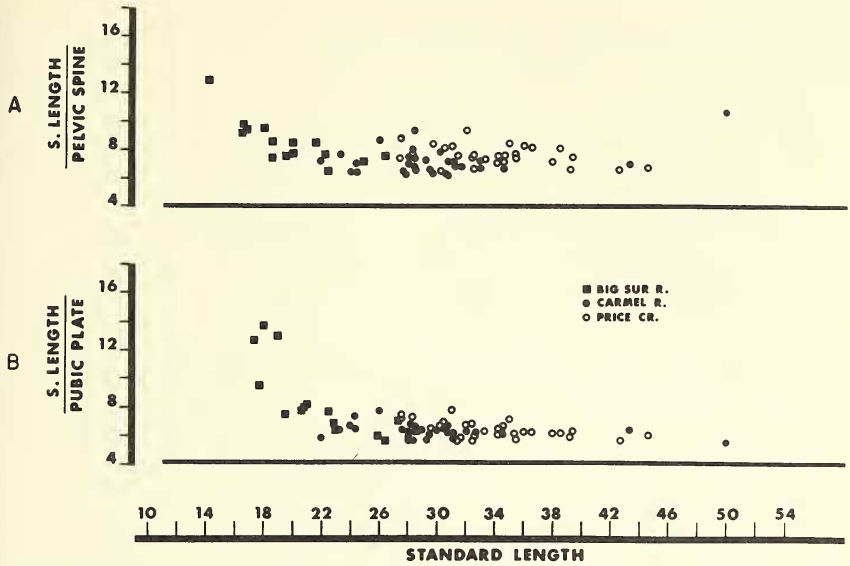

FIGURE 5. Pelvic-spine length (A), and pubic-plate length (B), vs. standard length.

long-spined group by pubic-plate length. The short-spined group, composed of the Alamo and Piru creek, Santa Clara and Mohave river, and Arroyo Trabuco samples, shows varying degrees of overlap with the northern group, with the latter sample being the most extreme.

The character index delineates three principal groups, the most northern of which is composed only of the San Lorenzo River sample (Fig. 4c). The second group includes samples from Carmel, Big Sur, Nacimiento, and San Antonio rivers, and Toro, Morro, San Luis Obispo, Arroyo Grande, Price, and Salsipuedes creeks, and the Ventura River (range 5.0-7.6). The San Lorenzo sample shows 95% joint nonoverlap with the Carmel River sample, the northernmost location of the second group. There is a general trend towards a lower character index value in the more southerly populations. The Nacimiento and San Antonio river samples are most extreme in reduction of the index value but still are separable with 89% joint nonoverlap from Arroyo Trabuco. Sticklebacks from Alamo Creek and the Santa Clara and Mohave rivers comprise a third group which is distinct from the partially plated stickleback populations by well over 96% joint nonoverlap (CD = 1.97).

The Arroyo Trabuco sticklebacks, when tested with Kramer's (1956) modification of Tukey's w′ multiple range test using 18 sample means (Steele and Torrie, 1960), are significantly different from all other southern populations of plateless and partially plated sticklebacks. However, figure 4c indicates a closer affinity with the *G. a. williamsoni* populations in comparison with *G. a. microcephalus* populations. Alamo Creek and the lower Santa Clara River sticklebacks are not significantly different when tested by the above

TABLE 2
Percent of fertilized eggs for reciprocal crosses between *Gasterosteus aculeatus williamsoni* and *G. a. microcephalus*

	MALES			
	microcephalus	*williamsoni*	p(diag.)	p(rows)
			0.80	
microcephalus	$\bar{X}$=91 sd=0.09	$\bar{X}$=71 sd=0.34		0.05
FEMALES				
williamsoni	$\bar{X}$=67 sd=0.29	$\bar{X}$=88 sd=0.22		0.20
			0.60	
Combined control crosses	$\bar{X}$=90	sd=0.15		
			$p<0.01$	
Combined test crosses	$\bar{X}$=69	sd=0.31		

multiple range test, but they differ significantly from both the upper Santa Clara River and the Mohave River populations.

Experimental Investigations

The first postmating isolating mechanism, gamete incompatibility, was investigated by comparing the percent of eggs fertilized from each control and test cross. Control crosses showed higher mean fertilization values than the hybrid crosses (Table 2). Crosses using eggs from *G. a. microcephalus* females showed greater percent fertilization than the respective control and test crosses using females of *G. a. williamsoni.* Subsequent to fertilization, there were no statistically significant differences between control and hybrid crosses in embryo and larval mortality; t for paired comparisons .65 and .78 respectively (Table 3).

TABLE 3
Summary of embryo and larval mortality for reciprocal crosses between *Gasterosteus aculeatus microcephalus* and *G. a. williamsoni.* The results from 12° to 20°C incubation temperatures are included. The number of trials is shown in parentheses

	Fw. Mw.	Fw. Mm.	Fm. Mm.	Fm. Mw.
Total number hatched	136	131	362	304
Total number fertilized	158 (2)	191 (2)	491 (5)	405 (5)
Percent mortality	13.9	31.4	26.3	24.9
Surv. to one week	125	118	315	242
Percent mortality	8.0	10.0	13.0	20.4

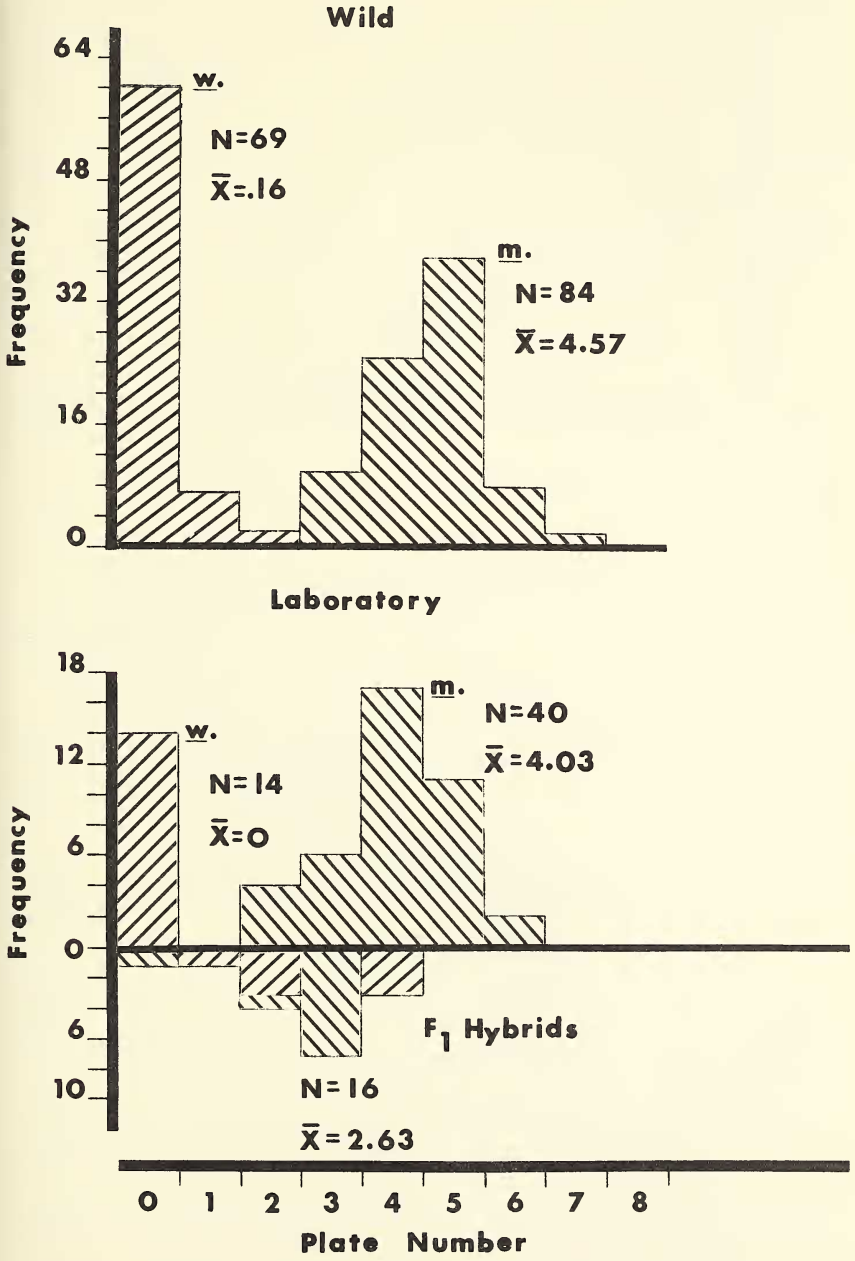

FIGURE 6. A comparison of lateral plate numbers of wild populations of *Gasterosteus aculeatus microcephalus* and *G. a. williamsoni* with laboratory reared controls and hybrids. The angle of hatching indicates the form of the female parent. *G. a. williamsoni* is shown by a positive slope, and *G. a. microcephalus* by a negative slope.

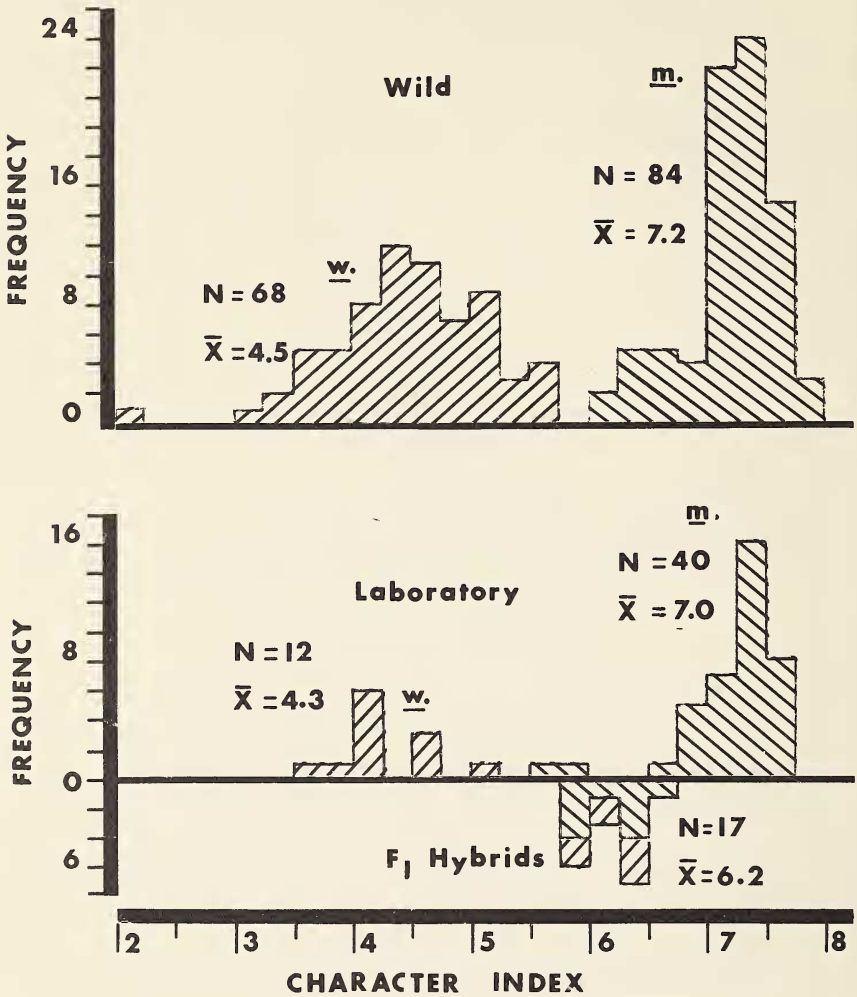

FIGURE 7. A comparison of character index values in wild populations of *Gasterosteus aculeatus microcephalus* and *G. a. williamsoni* with laboratory reared controls and hybrids. See legend for figure 6 for explanation of hatching.

A cross between a female *G. a. williamsoni* and a male *G. a. aculeatus* from Scott Creek, Santa Cruz County, California, showed 88.3% fertilization for the control and 50% fertilization for the test cross. No decrease in embryo or larval viability occurred, and the test cross was, if anything, more vigorous than the control.

All crosses had high embryo mortality at and below 10°C, and above 25°C, with the least mortality occurring between 15 and 20°C. Larval mortality of offspring from *G. a. microcephalus* females was lowest at 10°C and gradually increased with temperature from that point. Offspring from

G. a. williamsoni females remained fairly constant in percent mortality from 15 to 25°C.

Both hybrid plate counts and character index values indicate partial dominance of *G. a. microcephalus* over *G. a. williamsoni* (Figs. 6 and 7). Hybrid individuals cannot all be distinguished morphologically from parental controls although the character index is slightly more reliable than plate number.

Chromosome preparations were obtained from five *G. a. williamsoni,* six *G. a. microcephalus,* and two *G. a. aculeatus.* The diploid number in all three forms is 42 and morphology does not appear to differ from the karyotype of *G. aculeatus* published by Chen and Reisman (1970).

DISCUSSION

Three principally allopatric forms of *G. aculeatus* exist in central and southern California. Both Jordan and Hubbs (1925) and Miller and Hubbs (1969) felt the differences warranted subspecific recognition. The character index described in this paper allows morphological separation of the two southernmost forms with 96% joint nonoverlap. This is well above the arbitrary 90% suggested by Mayr (1969) for distinguishing subspecies. However, several populations show some degree of intermediacy.

G. a. microcephalus from the Nacimiento and San Antonio rivers resemble the more southern *G. a. microcephalus* populations in most of the characters studied. The number of river miles from the Pacific Ocean is much greater for these streams compared to other locations sampled, thus a reduction of gene flow with the coastal populations of *G. a. microcephalus* or with occasional stray *aculeatus-microcephalus* intergrades ("semi-armatus") is likely. This isolation is now enhanced by the frequent lack of water in the Salinas River during the summer months.

Natural selection must also play an important role to explain the divergence from the typical *G. a. microcephalus* phenotype of a similar latitude, assuming that the headwater populations are derived from sticklebacks on the coastal plain. If no selection were occurring, or if the characters studied were selectively neutral, one would not expect such a pronounced difference between the coastal and inland forms. Although the selective importance of predation has been demonstrated by several authors (e.g., Hoogland, Morris and Tinbergen, 1957; McPhail, 1969; Hagen and McPhail, 1970; and Hagen and Gilbertson, 1972), it is of questionable value in this instance. *Ptychocheilus grandis, Salmo gairdneri,* and *Cottus asper* were collected with *gasterosteus* in the Salinas headwaters. All three species are potential predators on *Gasterosteus. Cottus* was observed feeding on *G. a. williamsoni* in the laboratory. *Cottus asper* and *C. aleuticus* are also abundant in coastal streams from San Luis Obispo County northward. Consequently there is no apparent reduction in predation for the headwater populations of sticklebacks.

Further south, Miller and Hubbs (1969) listed intergrades from the Ven-

tura River by citing Rutter (1896: 248), but commented (p. 62) that their counts from the Ventura River and a tributary, "pertain to *G. a. microcephalus*." Rutter did not examine sticklebacks from the Ventura River. However, he did study 298 specimens from the Santa Clara River in Ventura County which have plate counts similar to those presented by Miller and Hubbs for the region near Fillmore. The Ventura River sticklebacks thus pertain clearly to *G. a. microcephalus*, although it is interesting that the gill raker counts are quite low compared to other *microcephalus* populations.

G. a. microcephalus also occurs below the Los Angeles plain in southern Orange County, northern San Diego County and northwestern Baja California. Plate numbers gradually increase for populations in northern San Diego County and in northwest Baja California (Miller and Hubbs, 1969).

Miller and Hubbs (1969) presented evidence for the introduce of *G. a. microcephalus* into Alamo Creek in 1940. Biological evidence of introduction is the increased variation in lateral-plate numbers, and also a tendency towards longer pelvic spines and pubic plates.

The Santa Clara River sample from the Los Angeles-Ventura County line also tends towards *G. a. microcephalus*. Lateral-plate counts of specimens collected near Fillmore (Miller and Hubbs, 1969) indicate an even greater shift towards *G. a. microcephalus*. The most obvious explanation is gene exchange with *G. a. microcephalus*.

Both *G. a. williamsoni* and supposed *G. a. microcephalus* x *williamsoni* intergrades have been introduced into the Mohave River (Miller and Hubbs, 1969). The high morphological variability of the Mohave sample is likely due both to the combined gene pools and to the breakdown of normalizing selection accompanying invasion of a new habitat. Sticklebacks were not previously found in the Mohave River, an endorheic stream (Hubbs and Miller, 1948).

Sticklebacks from Arroyo Trabuco, a tributary of San Juan Creek, also present taxonomic problems. Miller (1939) referred the San Juan Creek sticklebacks to *G. a. williamsoni* then later retracted his decision without assigning the form to *G. a. microcephalus* (Miller, 1960). Miller and Hubbs (1969) placed the form with *G. a. microcephalus* but suggested that a case could be made for calling it an intergrade. The character index suggests a closer affinity with *G. a. williamsoni,* but individual characters such as plate number, gill rakers, and pubic-plate length are intermediate. In the absence of a more complete study, it seems best to treat the Arroyo Trabuco-San Juan Creek sticklebacks as *G. a. williamsoni* x *microcephalus*, as suggested by Miller and Hubbs (1969).

Miller and Hubbs interpreted the morphology of the San Juan Creek and Arroyo Trabuco sticklebacks, as well as other partially plated sticklebacks further south, to southward movement of *G. a. microcephalus* during a glaciation. However, many features of the *G. a. microcephalus* phenotype are absent or reduced in the Arroyo Trabuco population. Consequently, even if intergradation did occur, gene frequencies have been markedly altered by

selective forces and/or dilution by backcrossing with the native form. The great importance of selective pressure on an understanding of character differences in *Gasterosteus* has been shown most recently by Hagen and Gilbertson (1972). A potential selective force may be the presence of *Fundulus parvipinnis* in San Juan and Arroyo Trabuco creeks (Miller, 1939), although direct predation, other than on eggs and larvae, seems unlikely. The extirpation of *G. a. williamsoni* from most of its former range has been tentatively linked by Miller (1961) to the introduction of *Gambusia affinis.*

It is interesting that the southernmost distribution of *Cottus asper* (Hubbs, 1921; McPhail and Lindsey, 1970) corresponds with the southernmost continuous distribution of *G. a. microcephalus* in the Ventura River. Both *C. asper* and *C. aleuticus* are abundant in coastal streams from San Luis Obispo County northward and could act as potential predators on sticklebacks. *C. asper* was observed feeding on *G. a. williamsoni* in the laboratory. South of the Ventura River the streams are generally without piscivorous fishes, with the exception of exotic salmonids, recently introduced centrarchids, and *Gambusia affinis.* The only potentially piscivorous fish listed by Culver and Hubbs (1917) for the Santa Ana system is *Salmo.* However, fluviatile rainbow trout prefer swift currents (Lewis, 1969), while *Gasterosteus* shows a preference for slower water. *Salmo* is not found in Soledad Canyon of the Santa Clara River. South of the hiatus in the distribution of *G. a. microcephalus* are several fishes, listed by Follett (1960), which could act as predators on sticklebacks. It is suggested that absence or reduction of predation may be a significant factor in the evolution of the *williamsoni* form. Clearly, the role of differential predation in the regulation of gene frequencies in stickleback populations of southern California needs to be investigated further.

No potential postmating isolating mechanisms between *G. a. microcephalus* and *G. a. williamsoni* were substantiated in the laboratory investigations, with the exception of a difference in percentage of fertilization. Field studies have shown that the breeding seasons overlap almost completely. Assuming a lack of preferential mating, the percentage difference in fertilization between the control and test crosses would not prevent gene flow between the forms, but would decrease the rate of exchange. However, if contact between the two forms were broken following an initial invasion, the percent difference in fertilization could constitute a significant selective force, reducing the frequency of the invading form. Selection against F_1 hybrids under natural conditions was not investigated, but laboratory produced hybrids developed normally. A high degree of ontogenetic compatibility is also indicated between *G. a. aculeatus* and *G. a. williamsoni,* reported in this study, and between *G. a. aculeatus* and *G. a. microcephalus* (Hagen, 1967).

Phenotypic dominance of *G. a. microcephalus* over *G. a. williamsoni* is shown by both meristic and morphometric characters. Consequently, F_1 *G. a. williamsoni* x *microcephalus* hybrids cannot be separated from *G. a. microcephalus* on the basis of plate number. Hagen (1967) demonstrated that *G. a. aculeatus* is dominant over *G. a. microcephalus* and Münzing (1963) showed

dominance of the fully plated European "trachurus" form over the partially plated "leiurus."

In conclusion, the evidence for postmating isolation between *G. a. microcephalus* and *G. a. williamsoni* is limited to a 21% differential in fertilization between intrasubspecific and intersubspecific crosses. Premating isolating mechanisms were not investigated.

Taxonomically the forms are distinct so that subspecific recognition is useful. However, the trinomial is used in the sense of Mayr (1963) in that it does not necessarily infer an evolutionary unit.

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